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THE STRUCTURE OF TYLOSIN[1,2]

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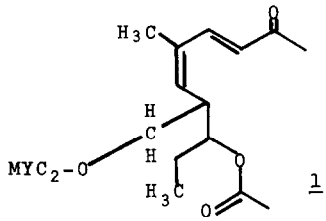
A partial structure[4] has been proposed for tylosin, a trisugar-containing macrolide antibiotic used in treatment of mycoplasmosis in poultry. The information presented in the earlier work is represented as structure 1.



1

-O-MYC$_3$

-O-MYC$_1$

-CHO

(-$C_9H_{16}O$)

The three sugars present in tylosin (pKa' = 7.1) can be cleaved sequentially by progressively more vigorous acid hydrolysis.[4,5] Removal of mycarose (MYC$_1$) yields desmycosin (pKa' = 8.0) as the macrolide portion, and O-mycaminosyl tylonolide (OMT) (pKa' = 8.0) is obtained by subsequent cleavage of mycinose (MYC$_2$). Removal of mycaminose (MYC$_3$), the amino sugar, results in destruction of the remainder of the molecule. The increase in pKa' values found in the first step of this hydrolysis has been noted with other macrolides[6] and can be taken as evidence for the direct attachment of mycarose to the mycaminose portion of the molecule as proposed previously for magnamycin.[6]

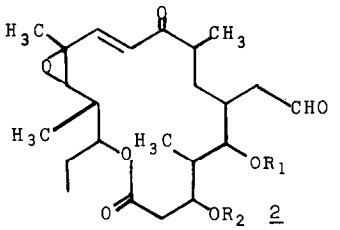
The availability of mass spectral data allows us to correct previously proposed empirical formulae for these compounds with the unambiguous values represented in Table I. As a consequence of these findings, the -$C_9H_{16}O$- portion of 1 becomes -$C_{10}H_{18}O$-. Evidence for the structure of this C_{10} unit (to which the aldehyde and two sugars must be attached) was obtained through oxidation of desmycosin with a mixture of potassium permanganate-sodium periodate, followed by digestion of the amino acid fraction from this reaction with potassium hydroxide under conditions described by Tsukiura, et al.,[7] for

TABLE I

Compound	Mol Wt	Emp Form.
Tylosin	915	$C_{46}H_{77}NO_{17}$
Desmycosin	771	$C_{39}H_{65}NO_{14}$
OMT	597	$C_{31}H_{51}NO_{10}$

cirramycin A_1 (2). A crystalline, $C_{13}H_{18}O_6$, unsaturated, acidic substance (3) was obtained and was identical in every respect to the compound 3 obtained from the degradation of cirramycin A_1 and kindly supplied to us by those workers.

$R_1=R_2=H$ or MYC_3



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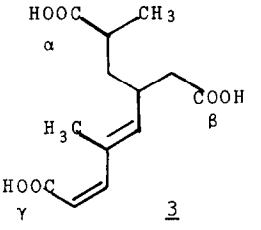
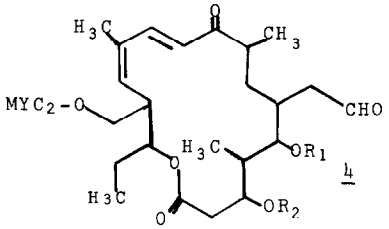


3

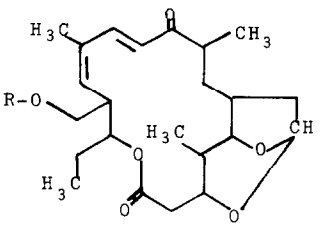
By analogy with all other known 16-membered ring macrolide antibiotics, it was reasonable to assume that the β-carboxyl of 3 originates from oxidation of the aldehyde moiety in desmycosin; however, either the α- or γ-acid function could represent the original lactone or ketone carbons. The answer to this uncertainty was obtained from studies of compound 5A and allows us to represent tylosin as structure 4.

Compound 5A and two additional compounds which provide further support for structure 4 are obtained in low yield as a result of the unexpected course of the reaction of desmycosin with chromium trioxide in pyridine. One of these latter substances is simply desmycosin in which one of the N-methyl groups of mycaminose has been oxidized to an N-formyl function:[8] ir 5.95 δ (amide carbonyl); pmr δ 7.9, (N-formyl proton), δ 2.8 (single N-methyl),(desmycosin: N-dimethyl, δ 2.5).



4

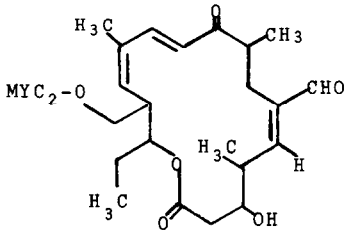
$R_1=R_2=H$ or MYC_3-MYC_1



5A: R=MYC_2

5B: R=H

The second compound, mycinsoyl anhydro nortylonolide (MAT), has lost the elements of mycaminose ($C_8H_{17}NO_4$) and a $-CH_2-$ unit, generating an α,β-unsaturated aldehyde: uv max 225 nm (15,600), 285 nm (20,700); pmr δ 6.8 (vinyl H), δ 9.46 (aldehyde); $C_{30}H_{46}O_{10}$ (M^+ = 566). This substance can be assigned structure 6[9] in which the remainder of the molecule, as determined by nmr comparison, is unchanged from desmycosin.



6

The third substance (5A) produced in this oxidation, a $C_{31}H_{48}O_{10}$ (M^+ = 580) compound, has also lost mycaminose but no longer shows an aldehydic proton in the pmr or additional unsaturation over that present in desmycosin. This compound, named mycinosyl tylonolide acetal (MTA) (5A), shows an additional proton as a doublet of doublets centered at δ 5.5, consistent with the existence of an acetal moiety and not present in other compounds discussed. Upon irradiation of the methylene region at δ 2.0, this δ 5.5 signal collapses to a singlet. The appearance of a doublet of doublets signal for the acetal C-H of 5A confirms that the aldehyde function in tylosin is directly attached to a methylene carbon despite the apparent singlet nature of the aldehydic proton in the pmr of 4. Similar observations have been noted with other closely related macrolides[7,10] in which derivatization of the aldehyde function results in a clear coupling pattern, revealing the presence of adjacent methylene protons. The pmr spectra of MTA exhibits two protons of a methylene group, representing the AB portion of an ABX system, centered at δ 2.5 (J_{gem} = 18 Hz, J_{vic} = 10.8 Hz) and δ 1.9 (J_{gem} = 18 Hz, J_{vic} = 1.2 Hz). The spectrum of these protons, which must be on a methylene group of a ring and next to a carbonyl,[11] remains unaltered upon sodium borohydride reduction of MTA and must therefore represent a methylene next to the lactone carbonyl group. Thus the tricarboxylic acid orientation is established as shown by structure 4.

An analogous tylonolide acetal (5B), $C_{23}H_{34}O_6$ (M^+ = 376), is obtained in very low yield from the neutral fraction separated during the preparation of OMT from desmycosin. Structure 5B was assigned after comparison of its physical properties with those of compound 5A.

Whether the location of the disaccharide portion (MYC_3- MYC_1) in tylosin is at R_1 or R_2 in 4 is not yet established. R_1 is suggested in order to explain the genesis of the additional double bond in MAT.

REFERENCES

1. Part 5 in a series on tylosin. Part 4 is reference 4.

2. R. B. Morin, P. V. Demarco, R. L. Hamill, and M. Gorman, Abstracts of Papers presented at the 9th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 27-29, 1969, Washington, D.C., Abstract 40, p. 17.

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4. R. B. Morin and M. Gorman, Tetrahedron Lett., 2339 (1964).

5. R. L. Hamill, M. E. Haney, Jr., M. Stamper, and P. F. Wiley, Antibiotics and Chemotherapy, 11, 328 (1961).

6. R. B. Woodward, Angew. Chem., 69, 50 (1957).

7. H. Tsukiura, M. Konishi, M. Saka, T. Naito, and H. Kawaguchi, J. Antibio., 22, 89 (1969).

8. C. Djerassi, A. Bowers, and H. N. Khastgir, J. Amer. Chem. Soc., 78, 1729 (1956).

9. An oxidation similar to that presumably involved in the conversion of 5A to 6 has been observed by others: U. Scheidegger, K. Schaffner, and O. Jeger, Helv. Chim. Acta, 45, 400 (1962).

10. S. Omura, A. Nakagawa, M. Otani, T. Hata, H. Ogura, and K. Furuhata, J. Amer. Chem. Soc., 91, 3401 (1969).

11. N. S. Bhacca and D. H. Williams, "Application of NMR Spectroscopy in Organic Chemistry", Holden-Day, Inc., San Francisco, California, 1964, p. 57, 63.